

Supplemental Information

Figure S1. Receptor Binding Properties of Human H10N8 HA

(A/Jiangxi-Donghu/346/2013).

Figure S2. Sequence Alignment of Full-length H10N8 HA with those from other H10 viruses.

Figure S3. Structural Comparison of HA-Avian Receptor Complexes from Human H10N8 and Avian and Human Pandemic H1N1 Viruses.

Figure S4. Structural Comparison of HA-Human Receptor Complexes from Human H10N8 and Human H1/H3/H5/H7/H10 Viruses.

Table S1. Glycans Imprinted on the Microarray.

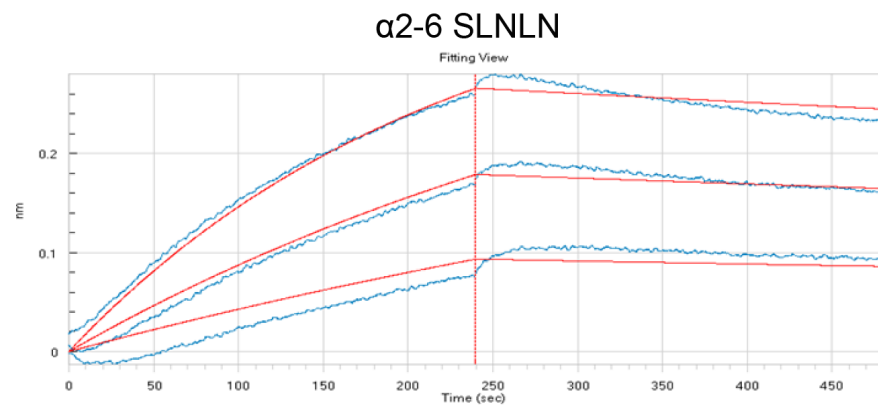
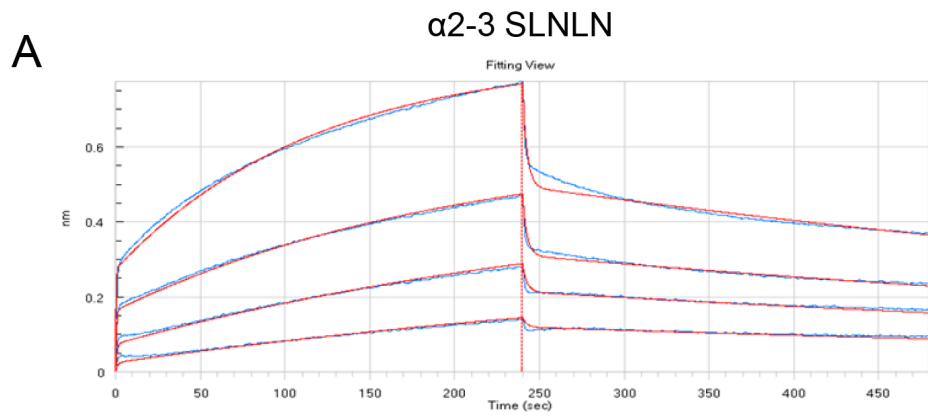
Table S2. Acknowledgements to GISAID Sequence Submitters.

Table S3. Data Collection and Refinement Statistics for H10 HA and Glycan Complexes

Table S4. Comparison of Residues Contributing to Receptor Binding in Human H10 HA with those in other HAs.

Supplemental Experimental Procedures

Supplemental References



B ☐ $\alpha 2-3$ Linked ☒ $\alpha 2-6$ Linked ☐ $\alpha 2-3$ & 2-6

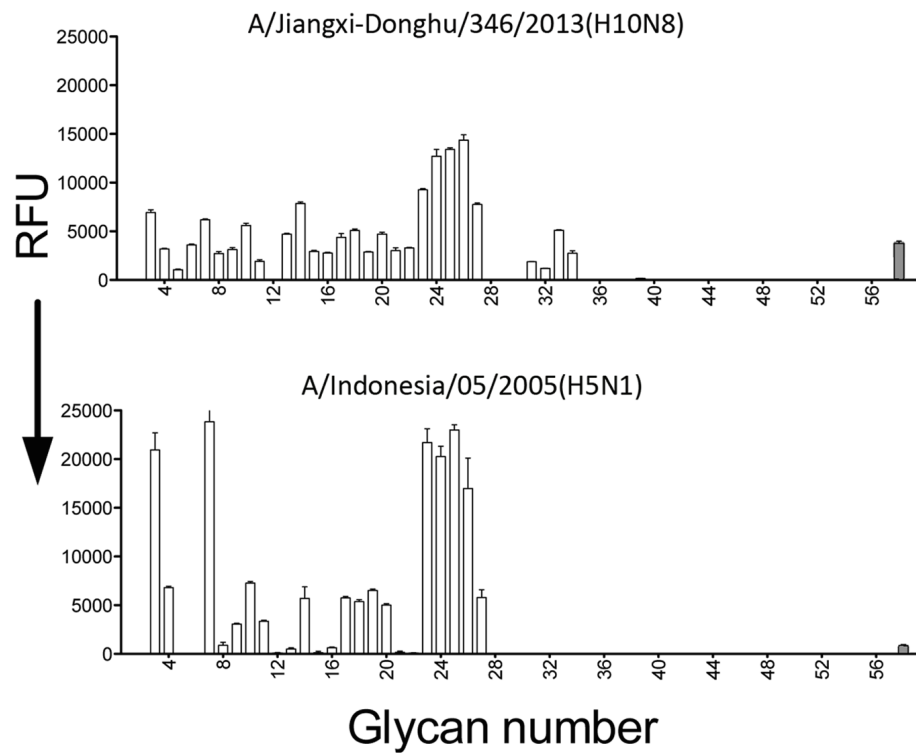


Figure S1, related to Figures 1B and 1C. Receptor Binding Properties of Human H10N8 HA (A/Jiangxi-Donghu/346/2013)

(A) Binding curves for α 2-3 SLNLN and α 2-6 SLNLN binding to the H10 HA, related to Figure 1B. Blue curves are the experimental trace obtained from bio-layer interferometry experiments and red curves are the best global fits to the data in Fig. 1B and used to calculate the K_d values reported in the text. Both binding curves was best fitted using the 2:1 heterogeneous ligand binding model (Concepcion et al., 2009), similar to the binding characteristics of human H7N9 HA to α 2-3 SLNLN and α 2-6 SLNLN (Xu et al., 2013).

(B) Glycan binding specificity of the recombinant H10 HA H5N1 (A/Indonesia/05/2005) HA produced in insect cells, using a sialoside glycan microarray, related to Figure 1C. The mean signal and standard error were calculated from four independent replicates on the array. Nonsialylated controls (#1 and 2), α 2-3 sialosides in white bars (#3-35), α 2-6 linked sialosides in black bars (#36-56), and mixed biantennary glycans containing both α 2-3 and α 2-6–linked sialosides in gray bars (#57 and 58). Glycans imprinted on the array are listed in Table S1.

A

Consensus sequence

A/Jiangxi/IPB13/2013 (H1ON8)

A/Jiangxi/IPB13a/2013 (H1ON8)

A/Jiangxi/IPB13b/2013 (H1ON8)

A/Jiangxi/IPB13c/2013 (H1ON8)

A/environment/Dongting Lake/Hunan/3-9/2007 (H1ON8)

A/environment/Dongting Lake/Hunan/3-9/2007 (H1ON8)

A/environment/Dongting Lake/Hunan/3-9/2007 (H1ON8)

A/environment/Dongting Lake/Hunan/3-9/2007 (H1ON8)

A/Eurasian coot/Germany/R411/2010 (H1ON8)

A/zuddy shelduck/Mongolia/1602/2010 (H1ON8)

A/quail/Italy/1117/1965 (H1ON8)

A/longtail duck/Maryland/295/2005 (H1ON8)

A/common scoter/Maryland/297/2005 (H1ON8)

A/northern pintail/California/44221-656/2006 (H1ON8)

A/mallard/Sweden/7/2003 (H1ON8)

A/mallard/Interior Alaska/6MP0758/2006 (H1ON8)

A/shearwater/Australia/2/1972 (H1ON8)

A/quail/Italy/1117/1965 (H1ON8)

A/northern shoveler/California/9235/2008 (H1ON8)

A/duck/Guangdong/E1/2012 (H1ON8)

A/mallard/Korea/1203/2010 (H1ON8)

A/shorebird/Delaware Bay/379/2008 (H1ON8)

A/zuddy turnstone/New Jersey/1148668/2004 (H1ON8)

A/American green-winged teal/Ohio/13OS1869/2013 (H1ON8)

A/mallard/Sweden/233/2002 (H1ON8)

A/mallard/Sweden/2823/2003 (H1ON8)

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A/velvet scoter/Mongolia/879V/2009 (H1ON8)

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A/surf scoter/Mongolia/878V/2009 (H1ON8)

A/velvet scoter/Mongolia/879V/2009 (H1ON8)

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.....G.I.R..
.....G.I.R..
.....G.I.R..
.....G.I.R..
.....S.....R..
.....S.....R..
.....S.....R..
.....S.....R..
.....S.....R..
.....V.T.....R.....T.....I.....R..
D.K..V.....R.....T.....K..D..
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.....LVL.....H.....P.....K.....K..D..
.....LVL.....H.....S.....K.....K..D..
.....I.....V.....H.....N.....K..D..
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.....V.....R.....T.....K.....K..D..
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.....R.....T.....K.....K..D..
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.....I.....V.....H.....N.....R..
.....V.....R.....R.....

60 70 80 90 100 110
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..K.....M.....V.....
..K.....M.....V.....
..K.....M.....V.....
..K.....M.....V.....
..S.....K.....I.....
..S.....K.....I.....
..S.....K.....I.....
..S.....K.....P.....I.....
R..SY.....R.....I.L.....V.E.....A.....I.....
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..N..Y.....VV.....D.S.....S.....
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..S..Y.....V.....D.S.....K.....
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..S..Y.....I.....V.....K.....
..S.....V.....I.....
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.....T.N.....
.....M.R.....P.....R.S.....G.T.....
D.....S.....
D.....S.....
D.....T.....
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D.....
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.....N.....S.....I.....
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A/Jiangxi/IPB13/2013(H10N8)

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.M..R..G
.M..R..G
.K..A..Y..S.T
.M..S..I
.SV..I
.SV..I
.S..I
.VM..I
.S..I
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.S..I
) S..I
.VM..I
.M..I
.M..I

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A/Jiangxi/IPB13/2013(H10N8)

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A/Jiangxi/IPB13/2013(H10N8)

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. . . . . RR . . . . . LI . . . . . L . . . . .
. . . . . RR . . . . . LI . . . . . L . . . . .
. . . . . RR . . . . . LI . . . . . L . . . . .
. . . . .
. . . . .
. . . . .
. . . . .
K . . . . . R . L . . . . . V . . . . .
. . . . .
E . . . . . K . R . L . . . . . VV . . . . .
K . . . . . L . . . . . VA . . . . .
K . . . . . L . . . . . VA . . . . .
K . . . . . L . . . . . VV . . . . .
. . . . .
K . . . . . L . . . . . VV . . . . .
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E . . . . . K . R . L . . . . . VV . . . . . K . . . . .
K . . . . . R . L . . . . . VV . . . . .
K . . . . . V . E . . . . .
. . . . .
K . . . . . L . . . . . VV . . . . .
K . . . . . L . . . . . VV . . . . .
K . . . . . R . L . . . . . VV . . . . .
K . . . . . R . . . . . H . . . . .
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A/Jiangxi/IPB13/2013 (H10N8)
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A/mallard/Sweden/2823/2003 (H10N8)
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A/mallard/Sweden/233/2002 (H10N8)
A/mallard/Sweden/2823/2003 (H10N8)
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A/velvet scoter/Mongolia/87B9/2009 (H10N8)

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A/Jiangxi/1PB13a/2013 (H10N8)
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210

220

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A/Seal/Sweden/SVA0824/2014(H10N7) MYKIVVIALLGAVKGLDKICLGHHAVANGTIVKTLTNEQEVTNATETV
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A/harbour_seal/Germany/1/2014(H10N7) ESTSLNRLCMKGRNKHDLGNCHPIGMLIGTPACDLHLTGTWDTLIERKNA
A/harbor_seal/Denmark/14-0561/2014-07(H10N7) ESTSLNRLCMKGRNKHDLGNCHPIGMLIGTPACDLHLTGTWDTLIERKNA
A/Seal/Sweden/SVA0546/2014(H10N7) ESTSLNRLCMKGRNKHDLGNCHPIGMLIGTPACDLHLTGTWDTLIERENA
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A/Sydney/2/2010(H10N7) -----
A/Mink/Sweden/3900/1984(H10N4) ESTSLNRLCMKGRSYKDLGNCHPIGMLIGTPACDLHLTGTWDTLIERKNA
A/mink/Sweden/1984(H10N4) ESTSLNRLCMKGRSYKDLGNCHPIGMLIGTPACDLHLTGTWDTLIERKNA

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A/Jiangxi-Donghu/346-1/2013(H10N8) IAYCYPGATVNEALRQKIMESGGINKISTGFTYSSINSAGTTTACMRN
A/Jiangxi-Donghu/346-2/2013(H10N8) IAYCYPGATVNEALRQKIMESGGINKISTGFTYSSINSAGTTTACMRN
A/Seal/Sweden/SVA0824/2014(H10N7) IAYCYPGATVNEALRQKIMESGGINKISTGFTYSSINSAGTTTACMRN
A/harbour_seal/Germany/1/2014(H10N7) IAYCYPGATVNEALRQKIMESGGINKISTGFTYSSINSAGTTTACMRN
A/harbor_seal/Denmark/14-0561/2014-07(H10N7) IAYCYPGATVNEALRQKIMESGGINKISTGFTYSSINSAGTTTACMRN
A/Seal/Sweden/SVA0546/2014(H10N7) IAYCYPGATVNEALRQKIMESGGINKISTGFTYSSINSAGTTTACMRN
A/Sydney/1/2010(H10N7) -----
A/Sydney/2/2010(H10N7) -----
A/Mink/Sweden/3900/1984(H10N4) IAYCYPGTTINEGALRQKIMESGGISKISTSTGFAYGSSINSAGTTTACMRN
A/mink/Sweden/1984(H10N4) IAYCYPGTTINEGALRQKIMESGGISKISTSTGFAYGSSINSAGTTTACMRN

A/Jiangxi-Donghu/346/2013(H10N8) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/Jiangxi-Donghu/346-1/2013(H10N8) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/Jiangxi-Donghu/346-2/2013(H10N8) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/Seal/Sweden/SVA0824/2014(H10N7) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/harbour_seal/Germany/1/2014(H10N7) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/harbor_seal/Denmark/14-0561/2014-07(H10N7) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/Seal/Sweden/SVA0546/2014(H10N7) GGNSFYAELKWLVSXKQGNFPQTNTYRNTDTAEHLIMWGIHHPSSSTQE
A/Sydney/1/2010(H10N7) -----
A/Sydney/2/2010(H10N7) -----
A/Mink/Sweden/3900/1984(H10N4) GGDSFYAEVVKWLVSXKQGNFPQTNTYRNTDTAEHLIIWGIHHPSSSTQE
A/mink/Sweden/1984(H10N4) GGDSFYAEVVKWLVSXKQGNFPQTNTYRNTDTAEHLIIWGIHHPSSSTQE

A/Jiangxi-Donghu/346/2013(H10N8) KNDLYGTQSLSISVGSSTYRNNFVVPVVGARPVQNGQSGRIDFHWTLVQPG
A/Jiangxi-Donghu/346-1/2013(H10N8) KNDLYGTQSLSISVGSSTYRNNFVVPVVGARPVQNGQSGRIDFHWTLVQPG
A/Jiangxi-Donghu/346-2/2013(H10N8) KNDLYGTQSLSISVGSSTYRNNFVVPVVGARPVQNGQSGRIDFHWTLVQPG
A/Seal/Sweden/SVA0824/2014(H10N7) KNDLYGTQSLSISVGSSTYRNNFVVPVVGARPVQNGQSGRIDFHWTLVQPG
A/harbour_seal/Germany/1/2014(H10N7) KNDLYGTQSLSISVGSSTYRNNFVVPVVGARPVQNGQSGRIDFHWTLVQPG
A/harbor_seal/Denmark/14-0561/2014-07(H10N7) KNNLYGTQSLSISVGSSTYQNNFVVPVVGARPVQNGLSGRIDFHWTLVQPG
A/Seal/Sweden/SVA0546/2014(H10N7) KNDLYGTQSLSISVGSSTYQNNFVVPVVGARPVQNGQSGRIDFHWTLVQPG
A/Sydney/1/2010(H10N7) -----
A/Sydney/2/2010(H10N7) -----
A/Mink/Sweden/3900/1984(H10N4) KNDLYGTQSLSISVGSSTYQNNFVVPVVRARPVQNGQSGRIDFHWTLVQPG
A/mink/Sweden/1984(H10N4) KNDLYGTQSLSISVGSSTYQNNFVVPVVRARPVQNGQSGRIDFHWTLVQPG
```


	250	260	270	280	290
A/Jiangxi-Donghu/346/2013(H10N8)	DNITFSHNGGLIAPSRVSKLIGRGLIQSDAPIDNNCESKCFWRGGSINT				
A/Jiangxi-Donghu/346-1/2013(H10N8)	DNITFSHNGGLIAPSRVSKLIGRGLIQSDAPIDNNCESKCFWRGGSINT				
A/Jiangxi-Donghu/346-2/2013(H10N8)	DNITFSHNGGLIAPSRVSKLIGRGLIQSDAPIDNNCESKCFWRGGSINT				
A/Seal/Sweden/SVA0824/2014(H10N7)	DKITFSHNGGLIAPSRVSKLIGRGLIQSEAPIDNSCESKCFWRGGSINT				
A/harbour_seal/Germany/1/2014(H10N7)	DKITFSHNGGLIAPSRVSKLIGRGLIQSEAPIDNSCESKCFWRGGSINT				
A/harbor_seal/Denmark/14-0561/2014-07(H10N7)	DKITFSHNGGLIAPSRVSKLIGRGLIQSEAPIDNSCESKCFWRGGSINT				
A/Seal/Sweden/SVA0546/2014(H10N7)	DNITFSHNGGLIAPSRVSKLIGRGLIQSDASIDNNCESKCFWRGGSINT				
A/Sydney/1/2010(H10N7)	-----				
A/Sydney/2/2010(H10N7)	-----				
A/Mink/Sweden/3900/1984(H10N4)	DNITFSHNGGRIAPSRVSKLVGRGLIQSEASIDNGCESKCFWRGGSINT				
A/mink/Sweden/1984(H10N4)	DNITFSHNGGRIAPSRVSKLVGRGLIQSEASIDNGCESKCFWRGGSINT				
	300	310	320	1	10
A/Jiangxi-Donghu/346/2013(H10N8)	RLPFQNLSPRTVGQCPKYVNRRSLMLATGMRNVPELIQGRGLFGAIAAGFL				
A/Jiangxi-Donghu/346-1/2013(H10N8)	RLPFQNLSPRTVGQCPKYVNRRSLMLATGMRNVPELIQGRGLFGAIAAGFL				
A/Jiangxi-Donghu/346-2/2013(H10N8)	RLPFQNLSPRTVGQCPKYVNRRSLMLATGMRNVPELIQGRGLFGAIAAGFL				
A/Seal/Sweden/SVA0824/2014(H10N7)	RLPFQNLSPRTVGQCPKYVNKKSLMLATGMRNVPELVQGRGLFGAIAAGFI				
A/harbour_seal/Germany/1/2014(H10N7)	RLPFQNLSPRTVGQCPKYVNKKSLMLATGMRNVPELVQGRGLFGAIAAGFI				
A/harbor_seal/Denmark/14-0561/2014-07(H10N7)	RLPFQNLSPRTVGQCPKYVNKKSLMLATGMRNVPELVQGRGLFGAIAAGFI				
A/Seal/Sweden/SVA0546/2014(H10N7)	RLPFQNLSPRTVGQCPKYVNKKSLMLATGMRNVPELMQGRGLFGAIAAGFI				
A/Sydney/1/2010(H10N7)	-----				
A/Sydney/2/2010(H10N7)	-----				
A/Mink/Sweden/3900/1984(H10N4)	KLFPQNLSPRTVGQCPKYVNKKSLMLATGMRNVPEIMQGRGLFGAIAAGFI				
A/mink/Sweden/1984(H10N4)	KLFPQNLSPRTVGQCPKYVNKKSLMLATGMRNVPEIMQGRGLFGAIAAGFI				
	20	30	40	50	60
A/Jiangxi-Donghu/346/2013(H10N8)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRLIEKTN				
A/Jiangxi-Donghu/346-1/2013(H10N8)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRLIEKTN				
A/Jiangxi-Donghu/346-2/2013(H10N8)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRLIEKTN				
A/Seal/Sweden/SVA0824/2014(H10N7)	ENGWEGMVDGWYGFRHQNAQGTGLAADYKSTQAAIDQITGKLNRIIEKTN				
A/harbour_seal/Germany/1/2014(H10N7)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRIIEKTN				
A/harbor_seal/Denmark/14-0561/2014-07(H10N7)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRIIEKTN				
A/Seal/Sweden/SVA0546/2014(H10N7)	ENGWEGMVDGWYGFRHNAQGTGLAADYKSTQAAIDQITGKLNRIIEKTN				
A/Sydney/1/2010(H10N7)	-----GKLNRLIEKTN				
A/Sydney/2/2010(H10N7)	-----AIDQITGKLNRLIEKTN				
A/Mink/Sweden/3900/1984(H10N4)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRLIEKTN				
A/mink/Sweden/1984(H10N4)	ENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITGKLNRLIEKTN				
	70	80	90	100	110
A/Jiangxi-Donghu/346/2013(H10N8)	TEFESIESEFSEIEHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Jiangxi-Donghu/346-1/2013(H10N8)	TEFESIESEFSEIEHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Jiangxi-Donghu/346-2/2013(H10N8)	TEFESIESEFSEIEHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Seal/Sweden/SVA0824/2014(H10N7)	TEFESIESEFSEIDHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/harbour_seal/Germany/1/2014(H10N7)	TEFESIESEFSEIDHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/harbor_seal/Denmark/14-0561/2014-07(H10N7)	TEFESIESEFSEIDHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Seal/Sweden/SVA0546/2014(H10N7)	TEFESIESEFSEIDHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Sydney/1/2010(H10N7)	TEFESIESEFSEIEHQIGNIINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Sydney/2/2010(H10N7)	TEFESIESEFSEIEHQIGNIINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/Mink/Sweden/3900/1984(H10N4)	TEFESIESEFSEIEHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
A/mink/Sweden/1984(H10N4)	TEFESIESEFSEIEHQIGNVINWTKDSITDIWTYQAE LLVAMENQHTIDM				
	120	130	140	150	160
A/Jiangxi-Donghu/346/2013(H10N8)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/Jiangxi-Donghu/346-1/2013(H10N8)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/Jiangxi-Donghu/346-2/2013(H10N8)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/Seal/Sweden/SVA0824/2014(H10N7)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/harbour_seal/Germany/1/2014(H10N7)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/harbor_seal/Denmark/14-0561/2014-07(H10N7)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/Seal/Sweden/SVA0546/2014(H10N7)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHACDDSCMESIRNNYDHS				
A/Sydney/1/2010(H10N7)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHKDDNCMESIRNNYDHT				
A/Sydney/2/2010(H10N7)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHKDDNCMESIRNNYDHT				
A/Mink/Sweden/3900/1984(H10N4)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHTCDDSCMESIRNNYDHS				
A/mink/Sweden/1984(H10N4)	ADSEMLNLYERVVRKQLRQNAEEDGKGCFEYHTCDDSCMESIRNNYDHS				
	170	180	190	200	210
A/Jiangxi-Donghu/346/2013(H10N8)	QYREEALLNRLNINPVTLSGGYKDIILWFSFGASC FVLLAVVMGLFFFCFL				
A/Jiangxi-Donghu/346-1/2013(H10N8)	QYREEALLNRLNINPVTLSGGYKDIILWFSFGASC FVLLAVVMGLFFFCFL				
A/Jiangxi-Donghu/346-2/2013(H10N8)	QYREEALLNRLNINPVTLSGGYKDIILWFSFGASC FVLLAVVMGLFFFCFL				

A/Seal/Sweden/SVA0824/2014 (H10N7)	QYREEALLNRLNINPLTLSSGYKDVLWFSFGASCFVLLAVVMGLVFFCL
A/harbour_seal/Germany/1/2014 (H10N7)	QYREEALLNRLNINPVTLSGGYKNVILWFSFGASCFVLLAVVMGLVFFCL
A/harbor_seal/Denmark/14-0561/2014-07 (H10N7)	QYREEALLNRLNINPVTLSGGYKDVLWFSFGASCFVLLAVVMGLVFFCL
A/Seal/Sweden/SVA0546/2014 (H10N7)	QYREEALLNRLNINPLTLSSGYKDVLWFSFGASCFVLLAVVMGLVFFCL
A/Sydney/1/2010 (H10N7)	QYREEALLNRLNISPVRLSSGYKDVLWFSFGASCFVLLAVIMGLVFF---
A/Sydney/2/2010 (H10N7)	QYREEALLNRLNISPVRLSSGYKDVLWFSFGASCFVLLAVIMGLVFF--
A/Mink/Sweden/3900/1984 (H10N4)	QYREEALLNRLNINSVKLSSGYKDIIILWFSFGASCFVLLAAVMGLVFFCL
A/mink/Sweden/1984 (H10N4)	QYREEALLNRLNINSVKLSSGYKDIIILWFSFGASCFVLLAAVMGLVFFCL
220	
A/Jiangxi-Donghu/346/2013 (H10N8)	KNGNMRTCTICI
A/Jiangxi-Donghu/346-1/2013 (H10N8)	KNGNMRTCTICI
A/Jiangxi-Donghu/346-2/2013 (H10N8)	KNGNMRTCTICI
A/Seal/Sweden/SVA0824/2014 (H10N7)	KNGNMRTCTICI
A/harbour_seal/Germany/1/2014 (H10N7)	KNGNMR-----
A/harbor_seal/Denmark/14-0561/2014-07 (H10N7)	KNGNMRTCTICI
A/Seal/Sweden/SVA0546/2014 (H10N7)	KNGNMRTCTICI
A/Sydney/1/2010 (H10N7)	-----
A/Sydney/2/2010 (H10N7)	-----
A/Mink/Sweden/3900/1984 (H10N4)	KNGNMQCTICI
A/mink/Sweden/1984 (H10N4)	KNGNMQCTICI

Figure S2, related to Figure 2A. Sequence Alignment of Full-length H10N8 HA with those from other H10 Viruses.

(A) Alignment of H10N8 HA Sequences Deposited in NCBI Influenza Virus Database. [Note: A/Jiangxi/IPB13/2013 (H10N8) shown in the box is the A/Jiangxi-Donghu/346/2013(H10N8) strain used in our study].

(B) Alignment of HA sequences of the H10 viruses (H10N8, H10N7 and H10N4) isolated from mammals from GISAID's EpiFlu™ Database. The residues contributing to receptor binding are labelled in red (H3 numbering). The results also showed the residues in RBS are highly conserved. However, it is of note that there is a Q226L substitution in the H10N7 strain (A/harbor_seal/Denmark/14-5061-1lu/2014-07), and these H10N7 viruses are recently spreading efficiently among seals (Zohari et al., 2014).

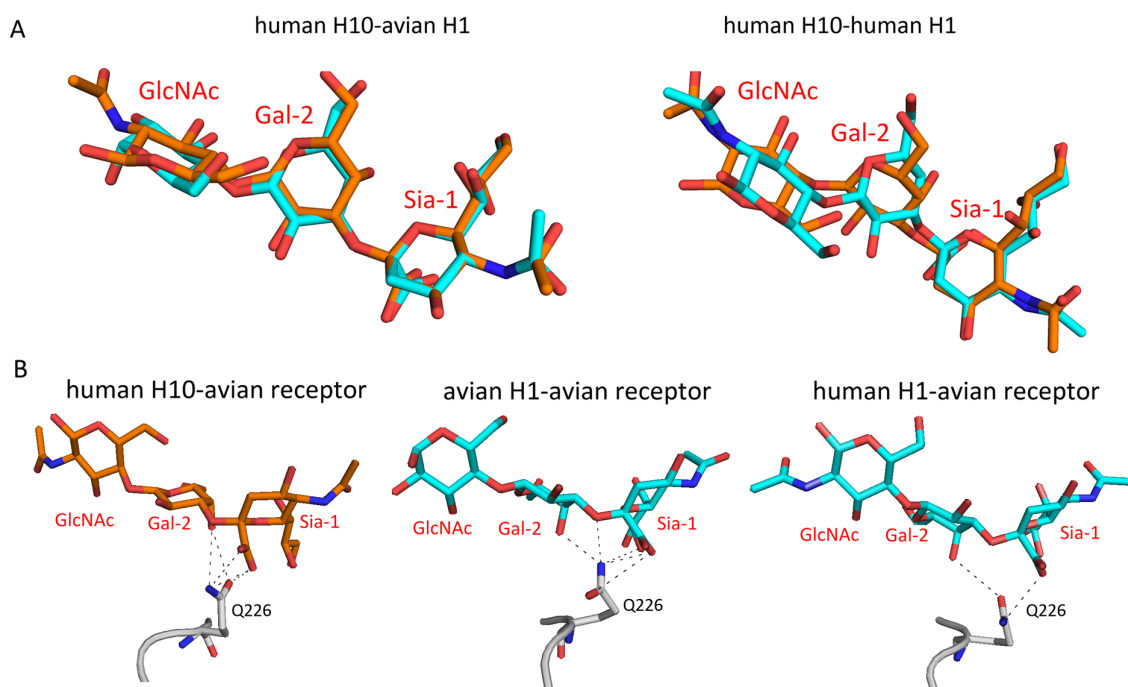


Figure S3, related to Figure 3B. Structural Comparison of HA-Avian Receptor Complexes from Human H10N8 and Avian and Human Pandemic H1N1 Viruses

(A) Superposition of 3'-SLN receptor analog from the H10 HA (orange) and avian and pandemic H1 HAs (cyan, PDB entries 3HTT and 3UBQ, respectively). The superposition indicates similar trans conformation of 3'-SLN in human H10 with avian H1, and conformational changes arising from rotation around the linkage between Sia-1 and Gal-2. The phi angle ($O6_{Sia}-C2_{Sia}-O-C6_{Gal}$) changes from $\sim 110^\circ$ in H10 and avian H1 structures to $\sim 70^\circ$ in human H1 structure). The superposition was done on Sia-1.

(B) The characteristic avian glutamine (Q226) forms five hydrogen bonds with Sia-1 and Gal-2 of 3'-SLN in human H10 similar to those in avian H1N1 (PDB entry 3HTT), while only two hydrogen bonds are formed in human H1N1 (PDB entry 3UBQ).

The H10 HA Q226 is conserved in all avian viruses and in human H1 viruses, but is L226 in human H2 and H3 influenza viruses, and a key determinant of receptor specificity. The interactions with the avian receptor in H10 resemble those of avian H1, and are distinct from interactions in human H1 HAs (Lin et al., 2009; Xu et al., 2012). In particular, Q226 forms five conserved hydrogen bonds with Sia-1 and Gal-2 in human H10 (Figure 3B), similar to those in the avian H1 complex, while only two hydrogen bonds are formed in the human H1 complex. Therefore, the preference of human H10 HA for avian receptors is closely associated with the avian glutamine like that in avian H1N1.

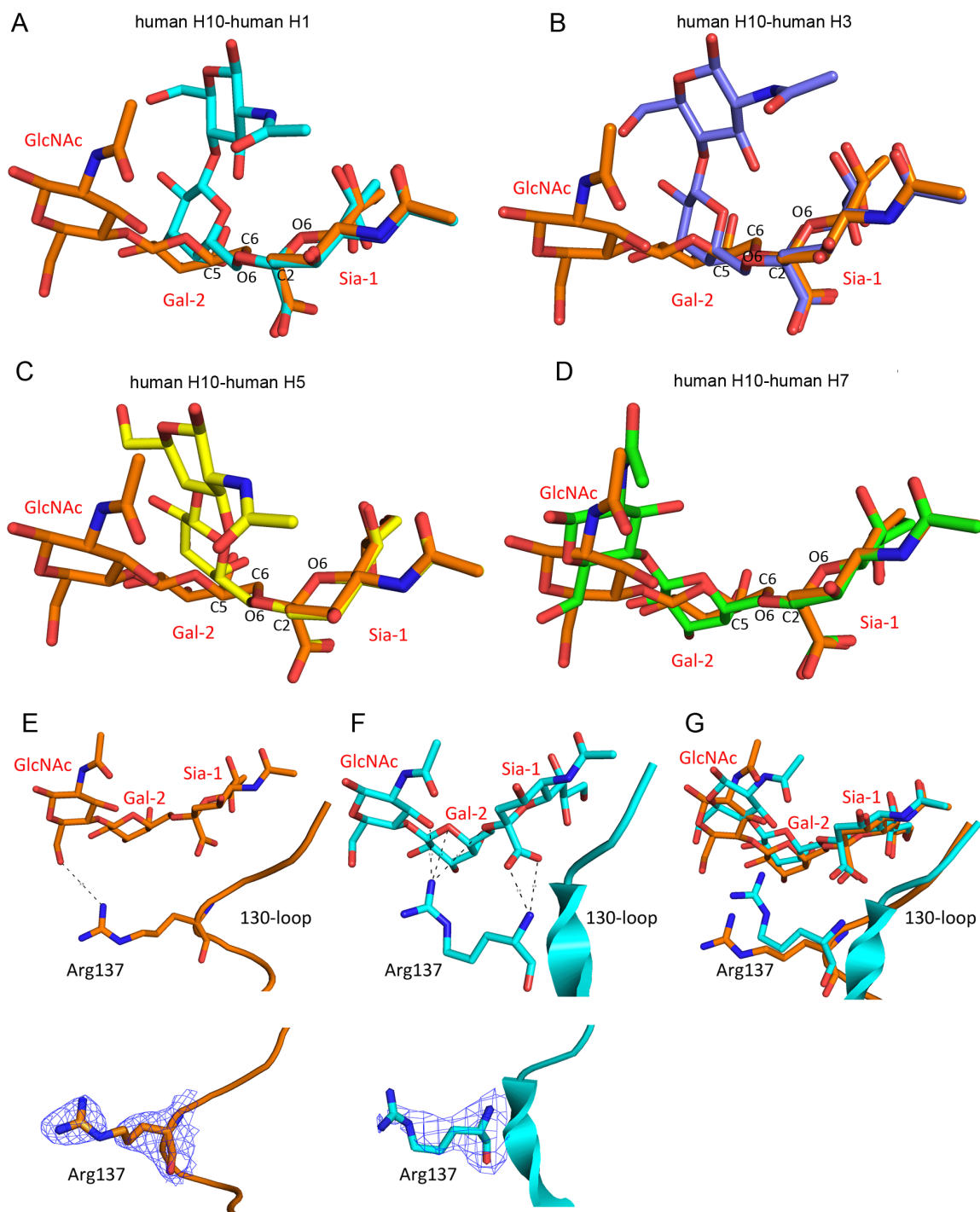


Figure S4, related to Figure 3C. Structural Comparison of HA-human Receptor Complexes from Human H10N8 and Human H1/H3/H5/H7/H10 Viruses

(A, B, C and D) Superposition of 6'-SLN receptor analogs from the H10 HA complex (orange) compared to that from pandemic H1N1 (cyan, PDB entry 3UBN) and H3N2 (C, blue, PDB entry 2YP3), ferret-transmissible H5N1 mutant (B, yellow, PDB entry 4BH3, Q226L) and human H7N9 (D, green, PDB entry 4BSC) complexes. The superposition

was done on Sia-1. The human receptor orientation in H10, especially the angle of rotation around the Gal-2 C6-C5 bond, is notably different from those in human H1/H3 and ferret-transmissible H5 complexes, but very similar to that in human H7 complex. The receptor in H10 also has slightly different phi angles ($O6_{Sia}-C2_{Sia}-O-C6_{Gal}$) from those in H1/H3/H5 complexes (from 41° in H10 to 71°, 71°, 61° and 58° in H1/H3/H5/H7, respectively).

(E, F and G) Structural comparison of HA-human receptor complexes of human H10N8 with the recently reported human H10 structure. In our structure (E, orange), Arg137 has good electron density and only very weak interaction (3.9 Å) with GlcNAc of the receptor. In another recently reported H10 HA structure (F, cyan, PDB entry 4D00), Arg137 interacts with all three sugars of the human receptor (the distances within 4 Å are labeled), but lacks electron density for Nε-1 and Nε-2. Arg137 moves closer to the human receptor in the recent reported H10N8 structure, although the receptor conformation is similar in both structures (G).

Table S1, related to Figure 1C and Figure S2. Glycans Imprinted on the Microarray. In these glycan structures, purple diamonds represent NeuAc, yellow circles represent Gal, blue circles represent Glc, green circles represent Man, yellow squares represent GalNAc, blue squares represent GlcNAc, red triangles represent Fuc and light blue diamonds represent NeuGc.

Table S2, related to Figure 2A and Figure S2. Acknowledgements to GISAID Sequence Submitters. We gratefully acknowledge the authors, originating and submitting laboratories of the following sequences that we obtained from GISAID's EpiFlu™ Database. All submitters of data may be contacted directly via the GISAID website www.gisaid.org

HA Segment ID	Isolate name	Originating Lab	Submitting lab	Authors	Country
EPI497477	A/Jiangxi-Donghu/346/2013	n/a	WHO Chinese National Influenza Center	Rongbao,Gao; Shumei,Zou; Xiang,Zhao; Lei,Yang; Dayan,Wang; Yuelong,Shu	China
EPI530256	A/Jiangxi-Donghu/346-1/2013	South China Agricultural University	South China Agricultural University	Wenbao,Qi; Xianfeng,Zhou; Lihong,Huang; Huanan,Li; Qian,Li; Ming,Liao; Mingbin,Liu.	China
EPI530534	A/Jiangxi-Donghu/346-2/2013	South China Agricultural University	South China Agricultural University	Wenbao,Qi; Xianfeng,Zhou; Lihong,Huang; Huanan,Li; Qian,Li; Ming,Liao; Mingbin,Liu.	China
EPI547696	A/Seal/Sweden/SVA0824/2014	Swedish Museum of Natural History	National Veterinary Institute	Siamak,Zohari; Aleksija,Neimanis; Tero,Härkönen; Charlotta,Moraeus; Jean-Francois, Vallachare	Sweden
EPI545212	A/Seal/Sweden/SVA0546/2014	Swedish Museum of Natural History	National Veterinary Institute	Siamak,Zohari; Aleksija,Neimanis; Tero,Härkönen; Charlotta,Moraeus; Jean-Francois,Vallachare	Sweden
EPI544356	A/harbour seal/Germany/1/2014	Erasmus Medical Center	Erasmus Medical Center	Bodewes, Rogier; Bestebroer, Theo M.; Van der Vries, Erhard; Verhagen, Josanne H.; Koopmans, Marion P.; Fouchier, Ron A.M.; Wohlsein, Peter; Siebert, Ursula; Baumgärtner, Wolfgang; Osterhaus, Albert D.M.E.	Germany
EPI541474	A/harborseal/Denmark/14-5061-11u /2014-07	Technical University of Denmark	Technical University of Denmark	Krog, Jesper Schak; Hjulsager, Charlotte; Larsen, Lars E	Denmark
EPI259037	A/Sydney/1/2010	n/a	Other Database Import	Ratnamohan,V.M.; Dwyer,D.E.	Australia
EPI259038	A/Sydney/2/2010	n/a	Other Database Import	Ratnamohan,V.M.; Dwyer,D.E.	Australia
EPI169313	A/Mink/Sweden/3900/1984	National Veterinary Institute	National Veterinary Institute	n/a	Sweden
EPI240179	A/mink/Sweden/1984	n/a	Other Database Import	Feldmann,H.; Kretzschmar,E.; Klingeborn,B.; Rott,R.; Klenk,H.D.; Garten,W.	Sweden

Table S3, related to Figures 2B, 3B and 3C. Data Collection and Refinement Statistics for H10 HA and Glycan Complexes

	Apo	3'-SLN	6'-SLN
Data collection			
Beamline	SSRL11-1	SSRL12-2	SSRL12-2
Wavelength (Å)	0.97945	1.0716	1.0716
Space Group	P2	P2	P2
Unit cell parameters (Å)	a=64.0 b=242.9, c=70.3 β=111.2°	a=64.8, b=245.8, c=70.9 β=112.4°	a=64.0 b=245.4, c=71.2 β=112.4°
Resolution range (Å)	50.00-2.60 (2.64-2.60) ^a	50.00-3.31 (3.31-3.25)	50.00-2.85 (2.90-2.85)
Observations	274,246	86,794	108,611
Unique reflections	60,451 (2944)	31,215 (1499)	42,408 (2114)
Completeness (%)	98.6 (96.8)	95.9 (91.7)	89.0 (90.3)
I/σ(I)	13.7 (2.2)	8.3 (1.1)	14.6 (2.0)
CC _{1/2}	0.84	0.65	0.66
R _{sym} ^b	0.12 (0.51)	0.18 (0.72)	0.18 (0.72)
R _{pim} ^c	0.05 (0.24)	0.10 (0.44)	0.10 (0.40)
Redundancy	4.5 (4.2)	2.8 (2.1)	2.6 (2.5)
Refinement			
Resolution (Å)	50.00-2.60 (2.64-2.60)	50.00-3.31 (3.31-3.25)	50.00-2.85 (2.90-2.85)
No. reflections	60,407	31,163	41,748
R _{cryst} ^e /R _{free} ^f	0.19/0.23	0.20/0.26	0.25/0.28
No. atoms			
Protein	11438	11379	11334
Carbohydrate	112	222	162
Water	262		27
Wilson B (Å ²)	29	79	61
Average B value (Å ²)			
Overall	33	86	67
Ligand		114	82
R.m.s deviation from ideal geometry			
Bond length (Å)	0.012	0.005	0.004
Bond angle (°)	2.0	1.8	1.9
Ramachandran Plot (%)			
Favored	95.6	95.2	94.9
Outliers	0.2	0.1	0.3

^bR_{sym} = $\sum_{hkl} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$, where $I_{hkl,i}$ is the scaled intensity of the i^{th} measurement of reflection h, k, l , and $\langle I_{hkl} \rangle$ is the average intensity for that reflection.

^cR_{pim} = $\sum_{hkl} (1/(n-1))^{1/2} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$, where n is the redundancy.

^dR_{cryst} = $\sum_{hkl} |F_o - F_c| / \sum_{hkl} |F_o| \times 100$, where F_o and F_c are the observed and calculated structure factors.

^eR_{free} was calculated as for R_{cryst}, but on a test set of 5% of the data excluded from refinement.

^fCalculated using MolProbity (Davis et al., 2007)

Table S4, related to Figures 2A and 2C. Comparison of Residues Contributing to Receptor Binding in Human H10 HA with those in the HAs of Human Pandemic Viruses (H1N1, H2N2 and H3N2) and Avian Zoonotic Viruses H5N1 and H7N9 (H3 Numbering)

Strains	98	135	136	137	153	183	190	222	225	226	228
H10N8 A/Jiangxi-Donghu/346/2013	Y	T	T	R	W	H	E	Q	G	Q	G
H1N1 A/Brevig Mission/1/1918	Y	V	T	A	W	H	D	K	D	Q	G
H1N1 A/California/04/2009	Y	V	T	A	W	H	D	K	D	Q	G
H2N2 A/Singapore/1/1957	Y	G	S	R	W	H	E	K	G	L	S
H2N2 A/Japan/305/1957	Y	G	S	R	W	H	E	K	G	Q	G
H3N2 A/Hong Kong/1/1968	Y	G	S	N	W	H	E	W	G	L	S
H3N2 A/Finland/486/2004	Y	T	S	S	W	H	D	R	D	I	S
H5N1 A/Vietnam/CL12/2004	Y	V	S	S	W	H	E	K	G	Q	G
H5N1 A/Indonesia/5/2005	Y	V	S	S	W	H	E	K	G	Q	G
H7N9 A/Shanghai/02/2013	Y	A	T	S	W	H	E	Q	G	L	G
H7N9 A/Hangzhou/1/2013	Y	A	T	S	W	H	E	Q	G	I	G

Supplemental Experimental Procedures

Expression and Purification of the H10 HA for Crystallization. The H10 HA cDNA sequence (GISAID accession number: EPI497477) of A/Jiangxi-Donghu/346/2013(H10N8) was synthesized by Life Technologies (USA) and cloned into a pFastBac vector. Wild-type H10 HA was expressed in Hi5 insect cells with an N-terminal gp67 signal peptide, a C-terminal thrombin cleavage site, a foldon trimerization sequence, and a His₆-tag and expressed as described previously (Stevens et al., 2006). The expressed HA0 was purified via a His-tag affinity purification, dialyzed against 20 mM Tris-HCl pH 8.0, 100 mM NaCl, and then cleaved by trypsin (New England Biolabs, Ipswich, Massachusetts) to produce uniformly cleaved (HA1/HA2) and to remove the trimerization domain and His₆-tag. The digested protein was purified further by gel filtration chromatography using a Superdex-200 column (Pharmacia). The HA protein eluted as a trimer and was concentrated to 10 mg/ml.

Crystallization and Structural Determination of the H10 HA. Crystals of the H10 HA were obtained using the vapor diffusion sitting drop method (drop size 1 μ l) at 4 °C against a reservoir solution containing 0.2 M NaSCN, 20% (w/v) PEG 3350. Complexes of the HA proteins with receptor analogues were obtained by soaking HA crystals in the reservoir solution that contained glycan ligands in a final concentration of 5 mM. Prior to data collection, the crystals were flash cooled in liquid nitrogen. Diffraction data were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) (Table S3). Data were integrated and scaled using HKL2000 (Otwinowski and Minor, 1997). The initial H10 *apo* structure was solved by molecular replacement method using Phaser (McCoy et

al., 2005) with an H7 HA structure (PDB entry 4N5J) as a search model. The H10 HA *apo* structure was used as the starting model for structure determination of the H10 HA-glycan complex structures. Structure refinement was carried out in Phenix (Adams et al., 2002) and model building with COOT (Emsley and Cowtan, 2004). Final refinement statistics are summarized in Table S3.

Glycan Binding of Baculovirus-expressed HAs by Biolayer Interferometry.

Recombinant HA expression from the baculovirus expression system was buffer-exchanged into PBS buffer. Association of HA was measured on an Octet Red (ForteBio) against immobilized glycans on the sensors. 3'-SLNLN and 6'-SLNLN (V-Labs, Covington, LA) are biotinylated glycans and were immobilized using Super Streptavidin Biosensors (ForteBio). Association was measured for 300 seconds at 30°C by exposing the sensors to protein solution in 1x kinetic buffer (1x PBS, pH 7.4, 0.01% BSA, and 0.002% Tween 20) after which the sensors were exposed to 1x kinetic buffer for dissociation for 300 seconds at 30 °C.

Expression and Purification of H10 HA from Baculovirus Expression System for Glycan Microarray Analyses. The HA expression in baculovirus expression system was similar to the H10 HA expression for crystallization experiments. The expressed HA0s were purified through a His-tag affinity purification step and dialyzed against 20 mM Tris-HCl, 50 mM NaCl, pH 8.0 overnight at 4 °C. Proteins were concentrated to 1 mg/ml prior to binding assays.

Expression and Purification of H1, H5 and H10 HAs from Mammalian Expression System for Glycan Microarray Analyses. Codon-optimized H1, H5 and H10 encoding cDNAs (Genscript, USA) of A/Kentucky/UR06-0258/2007 (GenBank accession number:

CY028163), A/Viet Nam/1203/2004 (GenBank accession number: EF541403) and A/Jiangxi-Donghu/346/2013 (GISAID accession number: EPI497477) were cloned into the pCD5 expression as described previously (Xu et al., 2013). The HA proteins were expressed in HEK293S GnTI^{-/-} cells and purified from the cell culture supernatants as described previously (de Vries et al., 2010).

ELISA-like Glycan Binding of HA from Mammalian Expression. Purified, soluble trimeric HA was pre-complexed with horseradish peroxidase (HRP)-linked mouse anti-Strep-tag antibody (IBA) and with Alexa647-linked anti-mouse IgG (Life Biosciences) in a 4:2:1 molar ratio prior to incubation for 15 min on ice in 100 µl PBS-T, and incubated for 90 minutes on the micro well slides that contained six replicates of PAA-linked 3'-SLNLN and 6'-SLNLN (provided by the Consortium for Functional Glycomics). Slides were subsequently washed, by dipping, in PBS-T, PBS, and deionized H₂O (4 times each). Washed arrays were dried by centrifugation and immediately scanned for FITC signal on a Perkin-Elmer ProScanArray Express confocal microarray scanner. Fluorescent signal intensity was measured using Imogene (Biodiscovery) and mean intensity minus mean background was calculated and graphed using MS Excel. For each glycan, the mean signal intensity is calculated from 6 replicates spots. The highest and lowest signals of the 6 replicates are removed and the remaining 4 replicates are used to calculate the mean signal, standard deviation (SD), and standard error measurement (SEM)

Glycan Microarray Analysis of HAs Expressed in Insect and Mammalian Cells.

Insect and mammalian derived HA were analyzed on a glycan array essentially as above. Briefly, for mammalian cell derived HA, soluble trimeric HA was pre-complexed with

horseradish peroxidase (HRP)-linked mouse anti-Strep-tag antibody (IBA) and with Alexa647-linked anti-mouse IgG (Life Bioscience) in a 4:2:1 molar ratio . For insect cell derived proteins, a mouse anti-HIS antibody and an Alexa647-linked anti-mouse IgG (Life Bioscience) were combined in a 4:2:1 molar ratio. HA-antibody complex were incubated for 15 minutes on ice in 100 µl PBS-T, and incubated for 90 minutes on the microarray. Washing, scanning and analyses was done as described above. A list of glycans on the microarray is included in Table S1.

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